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# Antigenic Stimulation Specifically Reactivates the Replication of Archived Simian Immunodeficiency Virus Genomes in Chronically Infected Macaques

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**Human immunodeficiency virus/simian immunodeficiency virus (SIV) diversification is a direct consequence of viral replication and occurs principally in secondary lymphoid organs where CD4$^+$ T cells are activated and proliferate. However, the evolution of viral quasispecies may also be driven by various nonexclusive mechanisms, including adaptation to specific immune responses and modification of viral fitness. Analysis of viral quasispecies in SIV-infected macaques subjected to repeated antigenic stimulations allowed us to demonstrate transient expansions of SIV populations that were highly dependent upon activation of antigen-specific T cells. T-cell clones expanded in response to a particular antigen were infected by a specific viral population and persisted for prolonged periods. Upon a second stimulation by encounter with the same antigen, these specific genomes were at the origin of a new burst of replication, leading to rapid but transient replacement of the viral quasispecies in blood. Finally, longitudinal analysis of SIV sequence variation during and between antigenic stimulations revealed that viral evolution is mostly constrained to periods of strong immunological activity.**

Genetic variation of the AIDS viruses (human immunodeficiency virus [HIV] and macaque simian immunodeficiency virus [SIVmac]), a direct consequence of the error-prone reverse transcription in the absence of proofreading activity, has been extensively described both in patients and in experimentally infected macaques (35, 40, 47, 50). After an initial transmission bottleneck characterized by relative homogeneity of sequences in acute infection (14, 42), viral populations are constantly evolving during the course of disease development (8, 43). The relative importance of viral adaptation through increased viral fitness and resistance to host immune responses is still a matter of debate (2, 15, 18, 28, 30). However, spatial and temporal analyses of viral quasispecies demonstrated that viral evolution is not only related to adaptation but also results from stochastic events such as a antigen induced activation of viral transcription (3, 5, 6, 9, 10, 20, 41).

The long-term persistence of HIV/SIV is principally attributed to the presence of latently infected CD4$^+$ T cells in which the virus is transcriptionally silent (22, 27, 39). HIV/SIV replication is essentially confined to the immunocompetent structures of secondary lymphoid organs such as the germinal centers of the lymph nodes and spleen or the intestinal Peyer's patches (31, 32, 48, 49). In both HIV and SIV infection, antigenic stimulation has been shown to be the driving force of viral replication in vivo, a probable consequence of the NF-kB dependence of viral transcription (5, 6, 45, 46, 52). Latently infected CD4$^+$ T cells involved in antigen-specific immune responses transport the proviruses into these immunocompetent structures, where they become transcriptionally active after T-cell activation (1). This "Trojan horse" mechanism is at the origin of local viral replication, characterized by discrete quasispecies and strong founder effects (1, 5, 6). However, the influence of antigen induced T-cell turnover (activation expansion and death) supporting these replication bursts on global viral evolution was not explicitly addressed in those studies. In order to analyze the relationships between antigen-induced immune activation and the genetic variation of both local and systemic SIV populations, SIV genetic evolution was analyzed in chronically infected macaques subjected to multiple antigenic stimulations. DNA sequences were analyzed over time in peripheral blood mononuclear cells (PBMC) after antigenic stimulations and locally in experimentally induced delayed-type hypersensitivity (DTH) reactions.

## MATERIALS AND METHODS

**Experimental protocol.** Two rhesus macaques (Macaca mulatta) of Chinese origin, animals 94027 and 93015, were immunized by intradermal injections with incomplete Freund adjuvant against diphtheria toxin (DT; 5 Lf) and tetanus toxoid (TT; 20 Lf) with Pasteur DTPolio vaccine and against BCG ($10^7$ PFU) with Mérieux vaccine at weeks 0, 6, and 14. At week 31, both animals were infected by intravenous injection of 10 50% animal infectious doses ($AID_{50}$) of SIVmac251, a pathogenic isolate provided by R. C. Desrosiers and titrated in Chinese rhesus macaques (26). This was followed at weeks 104, 113, and 128 by an intradermal injection of the previous dose of DT, TT, and BCG, respectively. Blood samples were collected as described in Fig. 1 before and after each antigenic boost. Viral loads were determined by quantitative TaqMan PCR (Applied Biosystems) (13). Before sacrifice, at weeks 141 (macaque 93015) and 155 (macaque 94027), DTH reactions were generated in the left and right arms or in the left leg of the animals by intradermal injection of 3,000 IU of BCG purified protein derivative (PPD), 5 Lf of DT, or 20 Lf of TT in 0.1 ml, respectively. Injections were performed in duplicate at 24 and 12 h prior to sacrifice of the monkeys. Skin patches from the DTH sites, as well as one normal skin patch from the right leg (injected with glycerol alone), were removed, and dermal zones were dissected under a stereomicroscope.

**PCR amplifications and quasispecies analysis.** Total DNA extraction was performed on 50% of each dissected derma or on $10^6$ PBMC by using Masterpure extraction kit (Epicenter). DNA was used to amplify CD3γ and hypervari-

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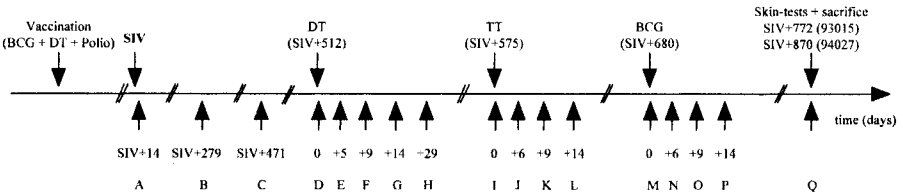


FIG. 1. Experimental protocol. Before being infected by SIVmac251, the animals were immunized against DT, TT or BCG until acquisition of a protective immunity. After SIV infection, each specific immunity was independently recalled. Before sacrifice, skin tests to DT, TT, or PPD were performed in the limbs of the animals. Blood samples were collected throughout the experiment, in particular during the immunization periods.

able regions 1 and 2 (V1V2) of the SIV env gene. The primers were CD3-out5′ (5′-GCCTGGCTGTCCTCATCCTGGCTATC), CD3-out3′ (5′-TGGCCTATG CCCTTTTGGGCTGCATCC), SIV-out5′ (5′-GAGGACGTATGGCAACTCT TTGAG), SIV-out3′ (5′-TAAGCAAAGCATAACCTGGAGGTG), SIV-in5′ (5′-TTGAGACCTCAATAAAGCCTTGTG), and SIV-in3′ (5′-CAAGATTCT TGGATAACAGAAGTG).

After an initial 10-min denaturation at 95°C, the PCR amplification protocol consisted of denaturation (95°C for 30 s), annealing (60°C for 30 s), and elongation (72°C for 2 min) for 35 cycles. Nested amplifications were performed on 10 ml of the first PCR using the same protocol. For each individual sample, two independent PCR amplifications were performed. PCR products from each amplification were purified in low-melting-point agarose, cloned into TOPO-2.1 TA vector (Invitrogen), and approximately 20 clones were sequenced for each individual sample. After alignment and indel coding (7), phylogenetic relationships between the different sequences were determined by the SplitsTree2 software (25). Statistical analysis of the evolution of SIV quasispecies in the different samples (chi-square test) was performed by using StatView software.

## RESULTS

**Quasispecies diversification is independent of initial plasma viral load.** SIV-negative macaques (animals 94027 and 93015) were extensively immunized against DT, TT, and BCG (Fig. 1). When immunizations were completed, as defined by specific serum antibody titers to each of the antigens considered in humans to confer protective immunity (>0.17 and 0.95 IU/ml for DT and TT, respectively) and a positive skin test to PPD, the animals were inoculated intravenously with 10 $AID_{50}$ doses of SIVmac251. SIV DNA quasispecies were serially analyzed over a 2-year period (Fig. 1). In both macaques, serum RNA viral load peaked at $10^7$ to $10^8$ copies/ml during the first 2 weeks of infection. Viral set points were subsequently established at $10^3$ and $10^4$ copies/ml in macaques 93015 and 94027, respectively (Fig. 2A). During the same period, no major variations in peripheral $CD4^+$ and $CD8^+$ counts and percentages were observed for both animals (Fig. 2B).

Viral quasispecies were analyzed in the inoculated viral stock, as well as in PBMC of both animals during the peak of viral load at 14 days postinfection. As expected, the viral stock used to inject the animals was heterogeneous. It was essentially composed of unique sequences, half of them could be grouped into two dominant forms, the others presenting original patterns of mutations (Fig. 2C). During acute SIV infection (day 14), highly homogeneous viral populations were observed in macaques 94027 and 93015 (Fig. 2C). Although a major sequence, accompanied by highly related minor sequences, represented 77% of the total DNA population detected during acute infection in macaque 93015, three sequences and their variants accounted for the near totality of the quasispecies detected at that time point in macaque 94027. None of the sequences identified during acute infection were detected in the viral stock. However, the sequences present at the peak of viremia were only transiently dominant. At 7 months postinfection (sample B), viral populations identified as dominant during acute infection were barely present. Although the dominant sequences were totally replaced by new sequences in macaque 93015, only one of the three dominant sequences remained detectable (<10%) in animal 94027, while two new groups of sequences represented 90% of the population (see below).

**Quasispecies evolution is not directly dependent on time.** After the initial phase of primary infection, the antigen-specific immunity of the animals was boosted by successive injections of DT, TT, and BCG (Fig. 1). A minimum of 2 months between each injection allowed the immune system to return to steady state. Viral DNA quasispecies were serially analyzed before and after each immunization. A total of 16 additional blood samples (B to Q, Fig. 1) were studied over the 2 years of follow-up for both animals.

In each individual sample, complex quasispecies composed of major and minor sequences were identified. In order to analyze viral evolution in the animals, major sequences and highly related sequences were identified as "motifs" differing from each other by at least five mutational events (Fig. 2C and D). Each motif was named by the main amino acid changes observed in this particular subset of sequences. For example, sequences detected at day 14 from macaque 93015 are called "RP," while motifs MNAT, LAST, and PVEK, respectively, represent sequences A-10, -1/-24, and -15 of day 14 sample from macaque 94027 (Fig. 2C and D). Fluctuations in the relative frequencies of these various motifs are presented in Fig. 3. In macaque 94027 (Fig. 3A), five motifs account for more than 60% of the sequences at any time point. However, their relative frequencies are highly variable, as they appear, expand to become the dominant motif, and contract to undetectable levels over the course of the experiment. For example, in macaque 94027, the motif LAST representing 100% of sample D and 54% of sample E totally disappears in subsequent quasispecies but subsequently rebounds to 95% of the sequences present in sample L; motif SKIP representing 89% of sample B totally disappears in subsequent quasispecies but represents again 77% of sample K and 36% of the sequences present in sample O. The much lower sequence diversity in macaque 93015 is also characterized by major fluctuations in the relative frequencies of the different motifs (Fig. 3B). Of note is the fact that, in macaque 94027, particular sequence motifs are present at immunization. For example, MNAT represents 82% of the sequences at day 6 postinjection of TT, MNAT and LAST are the major motifs in peripheral blood

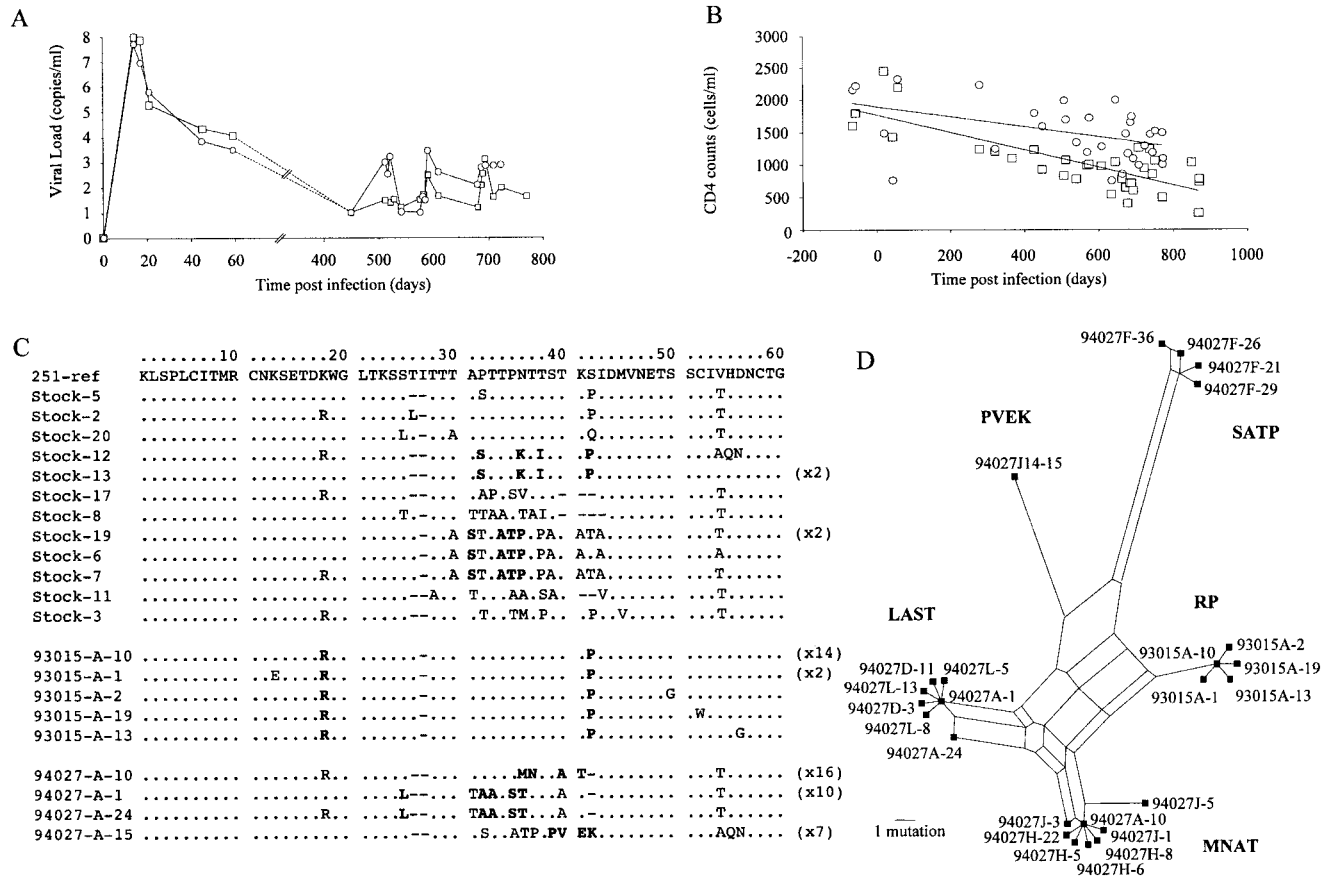


FIG. 2. SIV-V1 sequence evolution during acute infection. (A) Evolution of plasma viral load during acute and chronic infection. Plasma viral load was measured by real-time quantitative PCR for macaque 93015 (○) and macaque 94027 (□). (B) Evolution of peripheral blood $CD4^+$ counts in macaque 93015 (○) and macaque 94027 (□) during the course of the infection. (C) Sequence variation during acute infection. The diversity of viral quasispecies contained in the viral stock used to infect the animals (top panel), as well as viral populations present during the acute phase of the infection (sampled at 14 days postinfection) were analyzed for macaque 93015 (middle panel) and macaque 94027 (bottom panel). The hypervariable region V1 of the SIVmac env gene was PCR amplified, cloned, and sequenced. Approximately 20 clones from each sample were sequenced. Specific mutations defining the particular motifs are shown in boldface. (D) Identification of sequence "motifs" within peripheral viral populations. Phylogenetic analysis of representative example of SIVmac env V1 regions identified in the two animals over the course of the study was analyzed by using SplitsTree decomposition. Each motif is defined by a characteristic mutational pattern. A collection of 25 taxa is presented. The fit was 100%.

after DT immunization, and SATP characterizes viral quasispecies during BCG immune response.

The branch lengths in the phylogenic trees representing sequence diversity for any individual group of sequences (Fig. 3C and D) demonstrate that, within each pattern, viral evolution is minimal. In each of these trees, the central node corresponds to a unique sequence found at almost any time point of the experiment (9/10, 5/5, and 12/14 samples contained the central node sequence for motifs MNAT, LAST and RP, respectively). Many variants were generated during the 2 years of follow-up for all of the sequence patterns; however, fixation of particular mutations in subsequent samples was never observed, neither in macaque 94027 showing major variations in variant frequencies nor in the less-evolving quasispecies of macaque 93015.

**Antigen-specific reaction sites contain specific viral sequences.** At 12 and 24 h prior to sacrifice, skin tests were realized by intradermal injection of DT, TT, or PPD in each limb and right leg of both animals, respectively. Strong inflammatory reactions were observed at the PPD injection sites (>8 mm in diameter), while DT and TT induced less-intense reactions (~4 mm). Injection of glycerol into the forth limb did not result in any inflammatory reaction, demonstrating the specificity of the observed inflammation at the sites of antigenic stimulations. At sacrifice, the skin patches corresponding to the sites of antigen inoculation were sampled, and viral DNA quasispecies present in these inflammatory sites were analyzed.

As expected considering the relative lack of sequence diversity observed in macaque 93015, all of the skin tests contained the same dominant motif, precluding further analysis of the relationship between sequence population and antigenic stimulation in this animal. In contrast, as shown in Fig. 4, each skin test sampled 12 and 24 h after intradermal injection in macaque 94027 contained a particular SIV population composed of major sequences and highly related variants, resulting in a relatively homogeneous quasispecies in each antigen-specific patch. For each antigen, the dominant motifs in the 12- and 24-h skin patches was identical. The sequence motif found in the 12 h TT-specific immune site were identical to that of

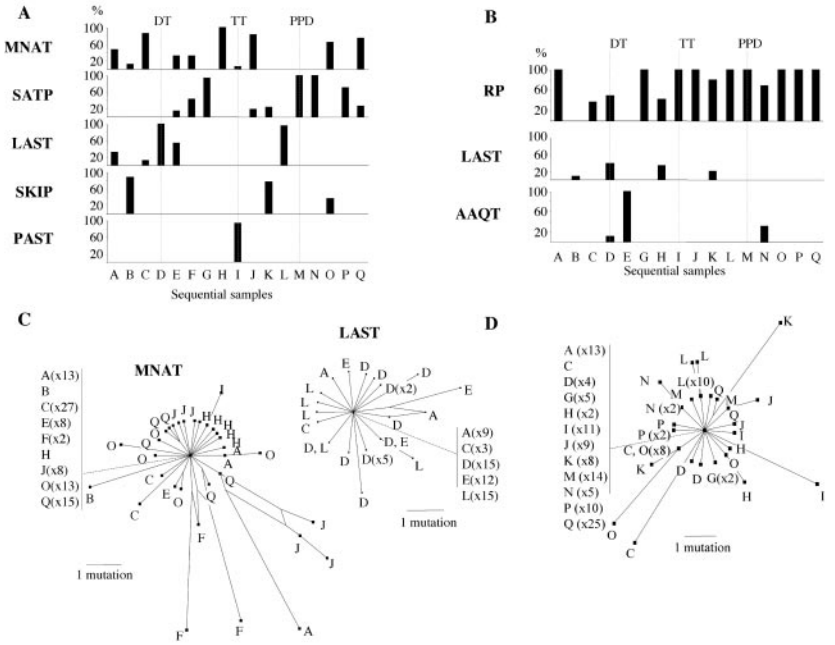


FIG. 3. Evolution of the V1 region of the SIVmac env gene during the course of the experiment. (A and B) The SIVmac env V1 region was PCR amplified, cloned, and sequenced from all blood samples (A to Q as defined in Fig. 1). Five and three dominant SIVmac env V1 motifs (MNAT, SATP, LAST, SKIP, PAST, RP, and AAQT) were identified in macaque 94027 and 93015, respectively. The relative frequencies of these motifs within proviral quasispecies are shown for each of the 17 samples analyzed for each animal. (C and D) Phylogenetic analysis of the dominant motifs identified in samples A to Q for macaque 94027 and 93015 were realized by using SplitsTree software. For each sample and motif, the dominant sequence is identified by the number of time this sequence was found in this sample. Collections of 130 and 149 taxa are presented in panels C and D, respectively. The fits were 100%.

DT-specific reactions but different ($P < 0.0001$) from that of PPD-specific ones (pattern MNAT in TT- and DT-specific reactions and pattern SATP in the PPD-specific skin test). More importantly, the exact same motifs were dominant in the peripheral blood immediately after the immunizations. The motif MNAT represents more than 30 and 80% of the circulating sequences at day 5 post-DT and -TT, respectively, while motif SATP characterizes post-PPD immunizations samples (Fig. 4B and C). This observation strongly suggests that, during each antigenic boost, viruses harboring a particular motif infected antigen-specific T cells. After intradermal injections, when antigen-specific memory T cells were recruited at the DTH sites, they locally introduced these particular viral sequences as proviral DNA. Finally, in TT- and PPD-specific antigenic reaction sites sampled at 24 h, the homogeneity of proviral populations is reduced, suggesting the infiltration of T cells presenting different antigenic specificities and harboring other proviral motifs in older inflammatory sites.

**The rate of evolution of viral sequence depends on immune activation.** The rapid fluctuation of HIV quasispecies and the frequent reappearance of SIV sequence motifs throughout the study allow estimating of rates of sequence variation in these animals. Considering that after the initial bottleneck and subsequent diversification of proviral populations each motif is independently evolving, it is possible for each motif to estimate the mutation frequency (number of mutational events per genome) between each pair of samples containing this motif. This was realized using the dominant sequence of the initial sample as a reference and plotted over time between the two particular samplings (Fig. 5A). Surprisingly, within sequence motifs, as well as looking at the whole population, no obvious relationship could be observed between mutation frequencies and time between samplings, again demonstrating that SIV sequence evolution is not directly dependent on time.

SIV sequences were analyzed within and between periods of experimentally induced immune activation. Some of these calculated mutation frequencies coincided with periods of intense immune activity after immunization. We thus calculated the fixation rates (mutations/genome/day) during these two types of periods (Fig. 5B). In both animals, the mutation rates during the immune activation periods were significantly higher than during periods of lower immune activity (median of 326 mutations/genome/day during immune stimulation periods compared to 4.4 between stimulations for mac93015 [$P < 0.001$] and 25 versus 0.8 for mac94027 [$P < 0.01$]), demonstrating that viral evolution essentially occurs during periods of strong immune activation.

## DISCUSSION

Despite considerable research, the relative importance of the different physiological factors at the origin of HIV and SIV genetic diversity remains debated (30, 35, 40, 50). Although sequence homogeneity generally characterizes acute infection, diversification is usually observed during the chronic phase of infection (8, 14, 42, 43). Whether the evolution of these quasispecies is dependent on selection pressure exerted by the host immune system, related to the relative fitness of viral popula-

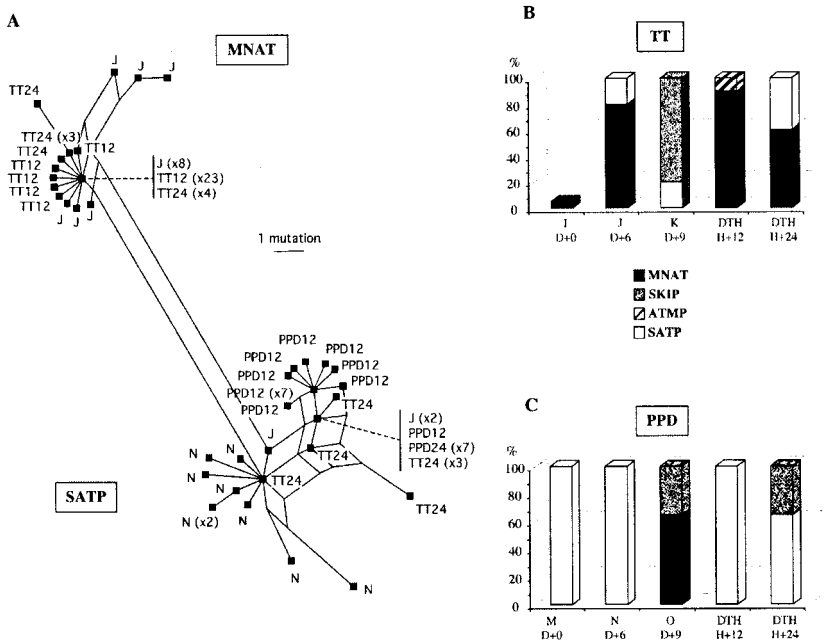


FIG. 4. Proviral populations in the skin tests. (A) Phylogenetic analysis of the dominant viral populations contained in TT- and PPD-specific skin tests and at day 6 after TT (J) and PPD (N) immunizations of macaque 94027. Samples were analyzed at 12 and 24 h after intradermal injections. A collection of 91 taxa is presented. The fits was 100%. (B and C) Relative frequencies of MNAT, SKIP, ATMP, and SATP motifs contained in TT- and PPD-specific skin tests and in peripheral blood at time of TT and PPD immunization (day 0, 6, and 9) for macaque 94027.

tions, or the consequence of random selection of proliferating mutants is still debated. In the present study, the extent of SIV variability was analyzed from acute infection and over a 2-year period in outbred rhesus macaques infected with a genetically complex preparation of SIVmac251. The influence of immune activation on sequence evolution was estimated by phylogenic analysis of the viral quasispecies developed during the immune responses initiated through experimental immunizations to several antigens.

After infection with a moderate amount (10 $AID_{50}$) of SIVmac251, both animals developed similar primary infection characterized by a peak of high viral load ($10^8$ copies per ml) at day 14, followed by the establishment of a viral set point at $\sim 10^4$ copies per ml. At the peak of acute infection, viral pop-

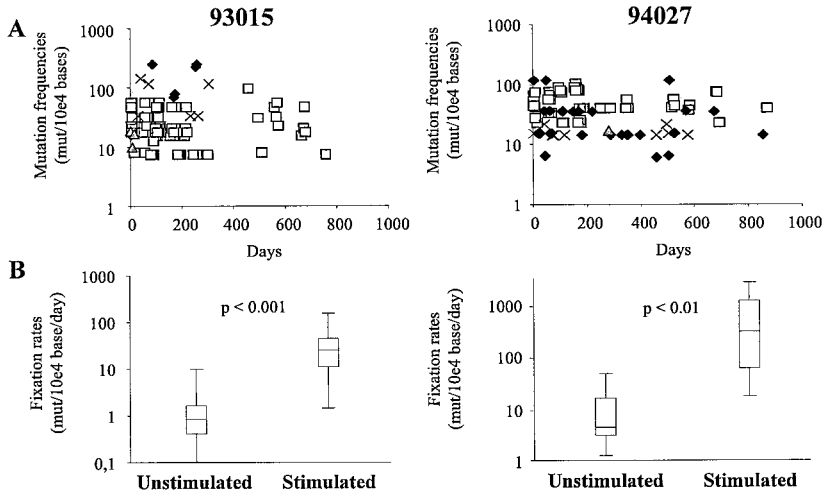


FIG. 5. The in vivo evolution rate of SIVmac depends on the activity of the immune system. (A) Mutation frequencies were calculated for the different motifs identified in the animals. For each pair of samples, the mutation frequency was calculated by using the dominant sequence as a reference. Motifs AAQT (◆), RP (□), PAST (△), and TAAST (×) are presented for macaque 93015 (left panel). Motifs MNAT (◆), SATP (□), SKIP (△), and LAST (×) are presented for macaque 94027 (right panel). (B) Fixation rates during (right) and outside (left) the antigenic stimulation periods. Mutation rates were calculated as the mutation frequencies divided by time between samplings for the various major motifs in macaque 93015 (left panel) and 94027 (right panel).

ulations were either homogeneous (macaque 93015) or composed of a few major motifs (macaque 94027). Very limited sequence variation was present within each motif. Moreover, the major sequences identified at day 14 in both individuals are different, and none of them were found within the 30 clones sequenced from the viral stock, suggestive of the existence of a "bottleneck" during the first days of infection. Such bottlenecks could be attributed to early infection of particular cells such as mucosal $CD4^+$ cells that have been shown to be the principal target of SIV infection and rapidly depleted during primary infection in both macaques and human (4, 48).

Previous studies demonstrated that viral populations sampled during acute infection are more diverse after intravenous inoculation than after intravaginal infection (16). Moreover, Greenier et al. identified similar sequences in five animals infected intravenously with the same viral stock (21). It is possible that the low inoculum used here mimics intravaginal route of infection, allowing the selection of rare variants during acute infection. However, similar variants (i.e., LAST and PAST), not identified in the stock, were found in both animals in later time points. It is thus highly probable that these variants were present at low frequency in the inocula. The reason for selecting a particular variant for the initial expansion remains unclear; however, our data are more in favor of a stochastic process than of a consequence of a better fitness since the initially expanded variants are totally replaced in later time points. Although the variants characterizing acute infection (LAST and MNAT) occasionally reappear in macaque 94027 (Fig. 3A), the RP motif becomes dominant in macaque 93015 in sample G, 526 days postinfection, during the DT-specific immune response (Fig. 3B).

Antigenic stimulation through the activation of specific T cells leads to proviral transcription. The lack of proofreading activity associated with reverse transcriptase ensures viral sequence diversification. However, viral quasispecies are highly compartmentalized in vivo as a consequence of localized replications at the sites of immune responses (11). This is true at the level of whole organs such as the lung in patients coinfected by M. tuberculosis (10, 34, 51), as well as in the germinal centers of secondary lymphoid organs (19, 20, 24). Moreover, antigen-specific T cells infiltrating DTH sites were shown to harbor highly homogeneous quasispecies (5). The compartmentalization of the viral populations in vivo was confirmed here in macaque 94027 by using three different antigens. Genetically distinct SIV populations were found in the skin tests directed against each antigen, while immune sites generated in response to similar antigenic stimulation do contain similar viral quasispecies. In the case of the anti-TT immune response, a major sequence (MNAT) is present in the skin tests 12 h after initiation of the DTH. This motif was found to be dominant in blood at day 6 after TT boosting, suggesting its association with anti-TT $CD4^+$ T cells (Fig. 4B). MNAT also represents the major circulating motif at the time of sacrifice. It is thus possible that the infiltration of the skin consists of both circulating and antigen-specific T cells. However, the absence of the second circulating major pattern (SATP) in the TT-specific skin test is in favor of the specificity, rather than serendipity, during the first 12 h of DTH reaction. In the skin test sampled at 24 h, although MNAT remains dominant, some SATP can be identified, suggesting that later during the DTH reaction, some nonspecific circulating T cells can be identified within the skin tests. In the case of PPD-specific reactions, cells containing sequences of the SATP subtype rapidly infiltrated the skin. This motif represents 100% at 12 h and remains dominant at 24 h (64%). This motif represented only 26% of the sequences observed in blood at that time point; however, it was dominant (100%) in sample N (day 6 after BCG immunization; Fig. 4C).

These data strongly suggest that antigen-specific T cells were infected during the secondary immune responses to TT and BCG with particular motifs present at that time, and expanded in periphery, together with the corresponding proviral motif, to reach a maximal frequency at day 6 postimmunization. At the end of the secondary immune responses, the frequencies of these particular proviral motifs returned to baseline, suggesting that these viruses were not particularly well adapted to their hosts. However, these motifs persisted in latently infected antigen-specific memory T cells and were selectively concentrated in the DTH reaction sites as a direct consequence of homing of memory T cells and sequestration of antigen-specific T cells.

In macaque 94027, viral quasispecies are characterized by frequent, rapid, and almost complete changes in the dominant circulating SIV motifs (Fig. 3). For example, motif PAST, representing 95% of the circulating proviruses in sample I could not be detected in the subsequent sample taken 6 days apart, motif SATP accounting for 100% in sample C drops to 0% in 5 days (sample D). A similar phenomenon was observed after DT immunization in macaque 93015; a complete switch between AAQT and RP motifs happened in as few as 9 days (between sample E and G). We have demonstrated that at least some of these changes are due to the expansion of SIV variants driven by antigen-specific immunity. It is thus probable that, at any time, the vast majority of the infected cells in peripheral blood correspond to cells effectively participating in ongoing immune responses. The speed of replacement of these peripheral proviruses fits with the short life span of effector memory T cells (12, 23, 33, 36, 37). This is also in agreement with the first phase of viral clearance after initiation of highly active antiretroviral therapy (29, 38).

In both macaques, peripheral DNA sequences are indeed rapidly changing throughout the course of the experiment; however, similar dominant sequences are often found in samples taken several months apart while they remained undetectable in all of the samples taken in between. Indeed, despite diversification within each motif during the periods of stimulation, the successive bursts of viral replication always originated from a common ancestor sequence. Accordingly, the dominant sequences for the various major motifs identified in the animals remain identical all over the course of the experiment. This "back to past" phenomenon seriously alters the possible conclusions concerning the speed of SIV evolution in these animals and highlight the necessity of multiple-sample analysis in the same individuals both to estimate the extent of sequence variation and to quantify viral replication in infected individuals. Using sequence diversification to estimate numbers of replication cycles may lead to underestimations.

Our findings also provide insights into the generation of long-term memory T cells. Two opposite scenarios are currently proposed: either antigen-specific effector T cells feed the

memory pool at the end of the immune response, or memory T cells differentiate early on during the proliferation process from recently activated cells (17, 44). Analysis of quasispecies evolution during antigen-specific activation of memory T cells allows investigation of this question. In the first alternative, one could expect the infecting viruses in this population to be highly diverse and to slowly diverge from the initial sequence. That was not observed in the animals analyzed here. In contrast, the observed recurrence of identical major sequences (Fig. 3C and D), even in macaque 94027, which demonstrated high sequence diversification throughout the experiment, supports the early differentiation hypothesis.

Overall, these results are consistent with a stochastic model of SIV evolution in vivo. During acute infection, as well as during the chronic phase, the dominant viral forms at any time point seem to depend more upon the infection of antigen-specific $CD4^+$ T cells by particular subtypes rather than upon selection of fitter viruses. Upon activation, the proliferation of these cells, as well as transmission of these particular viral subtypes to other antigen-specific cells, leads to the expansion of such sequence patterns that can become dominant in peripheral blood. However, in most cases, such dominance is only short-lived, the major forms being rapidly replaced—probably as a consequence of subsequent immune activation.

## ACKNOWLEDGMENTS

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